SURFACE ANTIGEN DYNAMICS IN THE SLIME MOLD, DICTYOSTELIUM DISCOIDEUM [1]

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The mechanisms by which cells in a morphogenetic system effect coordinated movements and specific associations constitute basic developmental problems that have been the subject of many investigations. That such mechanisms possibly involve forces similar to those existing between antigens and antibodies was recognized and elaborated by Tyler (1947, 1957), Weiss (1947), and Spiegel (1954a, 1954b). A recent comprehensive review of the problems involved in such morphogenetic processes as cell adhesion and cell migration has been presented by De Haan (1958) and need not be recapitulated here.

Morphogenetic expression in the slime mold, *Dictyostelium discoideum,* requires oriented movements of cells, a process which involves changes in shapes of the cells. The stimulus for the changes in shape may be an external one (via acrasin) in which large numbers of cells are affected from a common direction simultaneously (Bonner, 1947, 1950). Following the aggregation process the cells are organized into a pseudoplasmodium in which the cell surfaces are in intimate association with each other. Throughout morphogenetic movements the cells maintain contact with each other. Acquisition of the ability to adhere to one another implies a change in the nature of the surface of the cells. Consistent with this is the evidence obtained by Gregg (1956) that new antigens appear at the onset of aggregation.

It is the purpose of this study to investigate changes in the surface antigens of the cells from the beginning aggregation stage to the formation of the mature spores. Such a study might be expected to reveal not only the pattern of antigens during morphogenesis but something of the possible role of surface antigens in maintenance of the integrity of the pseudoplasmodium and in regulation of the course of cellular movement.

Methods and Materials

The slime molds were cultured according to the general methods employed by Bonner (1947) with the exception that the bacterial associate was *Aerobacter aerogenes* rather than *Escherichia coli.* The cultures were maintained at 22° C. during the growth phase. During the period of aggregation they were transferred to a 17° C. incubator, the lower temperature of which extended the migration and culmination phases.

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Preparation of injection-antigens

Migrating pseudoplasmodia were collected by cutting discs of nutrient agar bearing aggregates on the verge of migration. These discs were transferred to similar sized spaces in plates on non-nutrient agar (Gregg and Bronsweig, 1956). After the migrating pseudoplasmodia had accumulated on the non-nutrient surface they were collected individually with a hair loop and transferred into 1.0% NaCl.

The mature spores were collected by sweeping the tops of the mature sorocarps with a 3-mm. glass rod. The spores adhered to the glass rod and were transferred into 1.0% NaCl.

An antigen was also prepared by washing mature spores in 0.5% NaCl for 10–15 minutes. The spores were then centrifuged and the supernatant carefully removed to serve as an antigen.

These collecting procedures enabled migrating pseudoplasmodia, mature spore, and mature spore surface antigens to be obtained essentially free of *Aerobacter*. The antigens were stored in 1.0-ml. aliquots in a deep freezer at minus 15° C.

Each of the three types of antigens contained 0.2 mg. N/ml. Two rabbits each were injected with the three types of antigens. The injections were given every other day until a total of nine injections per rabbit had been given (0.5 ml. antigen/injection).

Preparation of antisera and normal (pre-injection) sera

The rabbits were bled by heart puncture five days following the final injection. The blood was allowed to stand for approximately two hours at room temperature before the clot was ringed, then placed in the refrigerator overnight before centrifugation. Complement was destroyed by heating the antisera at 56° C. for 30 minutes. The antisera were divided into 1.0-ml. aliquots and placed in the deep freezer at minus 15° C.

The normal (pre-injection) sera were obtained and prepared in the same manner as described for the antisera.

Preparation of absorbed antisera

The amoebae or spores were harvested from the culture plates by the methods described under the sections concerning the preparation of injection-antigens and test-antigens. However, in the absorption procedure all of the supernatant was removed from the packed cells. In a typical absorption, packed cells were mixed with the particular antiserum in the ratio of one volume of cells to one volume of antiserum. The preparation was allowed to absorb at room temperature for two hours followed by 22–24 hours at 10° C. The absorbed serum was then centrifuged in an electric micro-centrifuge (Microchemical Specialties Co.) at 9500 rpm for 15 minutes. The serum was then carefully removed from the packed cells and stored at minus 15° C. until desired.

Preparation of test-antigens for agglutination

These antigens were prepared by alternately washing and centrifuging (125 *g*) aggregating amoebae or spores twice in 0.5% NaCl. The resulting sedimented

cells were diluted with about one volume of 0.5% NaCl (approximately 2.5×10^8 cells/ml.) for use in agglutination tests. The cells were placed in agglutination chambers in 10.0-μl. volumes (Gregg and Trygstad, 1958). The antiserum or normal (pre-injection) serum was mixed with the cells in 10.0-μl. volumes with

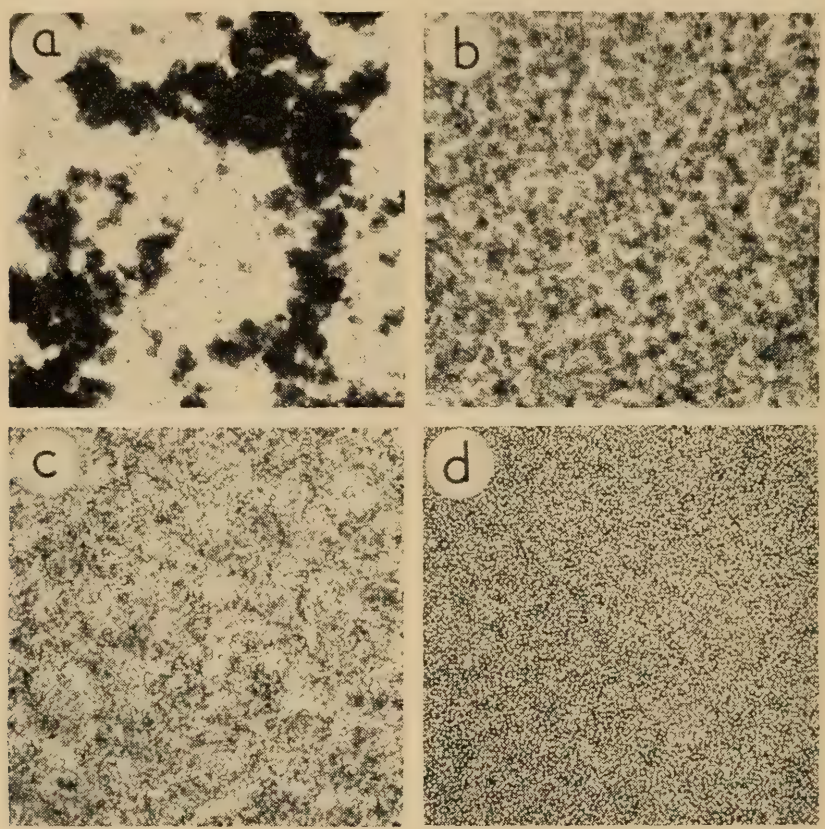

FIGURE 1a. *D. discoideum* amoebae + spore antiserum (magnification × 36). 1b. *D. discoideum* amoebae + spore normal serum (magnification × 36). 1c. *D. discoideum* spores + spore antiserum (magnification × 100). 1d. *D. discoideum* spores + spore normal serum (magnification × 100).

a small glass rod. The presence or absence of antibodies in the serum was detected by agglutination of the amoebae or spores to various degrees (Fig. 1a, b, c, d).

All antisera and normal (pre-injection) sera were tested on amoebae or spores independently. However, the results obtained from antisera produced in response

TABLE I

Antiserum titer obtained by agglutination of cells

Type of antiserum	Type of cell agglutinated	Highest dilution of antiserum
MA	Amoebae	1:80
SA	Amoebae	1:80
SSA	Amoebae	1:20

to the same type antigen or normal (pre-injection) sera have been pooled. The titers of the various sera were obtained by determining the least concentration of antiserum diluted with 1.0% NaCl, which would produce detectable agglutination of amoebae or spores (Table I).

Determination of the amounts of antigenic material extracted from the surfaces of the cells

For reasons which will become apparent it was necessary to determine quantitatively the amounts of antigenic material which may be extracted from the surfaces of the cells composing migrating pseudoplasmodia and the mature spore masses. These determinations were made in two ways as follows:

A. 1. Ten to fifty migrating pseudoplasmodia (non-nutrient agar migrants) or mature spore masses placed in 20 μl. of 0.5% NaCl.

2. Cell surface materials extracted by gentle stirring for 10–15 minutes.

3. Cells centrifuged 5 minutes at 4600 rpm in the micro-centrifuge.

4. Washed cells and surface wash analyzed separately for total N (Bruel *et al.*, 1946; Gregg, 1950) (Table IV).

B. 1. One hundred to five hundred migrating pseudoplasmodia or mature spore masses were placed in 100–500 μl. 1.0% NaCl.

2. Cell surface materials extracted by gentle stirring for 10–15 minutes.

3. Cells centrifuged 15 minutes at 9500 rpm in the micro-centrifuge.

4. The surface wash was removed and saved for analysis.

5. The washed cells were analyzed for total N.

6. The volume of each surface wash from each type of cell was adjusted such that $\frac{\mu\text{l. surface wash}}{\mu\text{g. N of washed cells}} = \text{unity}$. This dilution procedure resulted in the production of surface wash in volumes proportional to the number of cells (total N) from which it was extracted.

7. The surface washes were then subjected to conventional procedures to determine optimum proportions with various antisera in precipitin tests.

8. Fifty μl. of each surface wash and the optimum amount of each antiserum, determined in step 7, were mixed in 3 mm. × 40 mm. test tubes and allowed to incubate for 1–2 hours at room temperature. After incubation in a refrigerator overnight the precipitates were centrifuged at 9500 rpm for 15 minutes in the micro-centrifuge. The precipitates obtained were washed once with 1.0% NaCl and analyzed for total N (Table V).

Results

Migrating pseudoplasmodia-antiserum (MA)

The MA effected maximal agglutination of both amoebae and spores (Table II). It should be noted here, however, that spores do not exhibit the massive type of agglutination obtained with amoebae. The maximal agglutination in spores amounts to an easily detectable regular pattern of small clumps of spores, but never the large masses of drastically clumped cells as observed when agglutinating amoebae.

Migrating pseudoplasmodia-antiserum absorbed with amoebae (MAa)

The MAa did not agglutinate amoebae or spores (Table III).

Migrating pseudoplasmodia-antiserum absorbed with spores (MAs)

The MAs did not agglutinate amoebae or spores (Table III).

Migrating pseudoplasmodia normal (pre-injection) serum (MN)

The MN used as controls in all agglutination tests along with MA, MAa, and MAs, did not effect agglutination of either amoebae or spores (Tables II, III).

Mature spore-antiserum (SA)

The SA agglutinated both amoebae and spores to the degree designated as maximum for each type of cell (Table II) (Fig. 1a, c).

TABLE II

Degree of agglutination effected by various antisera and normal (pre-injection) sera on amoebae and spores

+ = maximum agglutination; ± = minimal agglutination; and − = no agglutination. All sera were diluted to one-half of full strength as a result of the addition of amoebae. The numbers opposite the degree of agglutination values refer to the number of times a particular pattern was observed.

	Type of sera	Amoebae Degree of agglutination			Spores Degree of agglutination*		
		+	±	−	+	±	−
Antisera	Migrating	49				26	
	Spore	40				24	
	Spore surface	21				13	
Normal sera	Migrating			48			24
	Spore			39			22
	Spore surface			19			15

* In this table and Table III, ± represents the maximal degree to which spores may be agglutinated.

Mature spore-antiserum absorbed with amoebae (SAa)

The SAa failed to agglutinate amoebae, but effected maximal agglutination of spores (Table III).

Mature spore-antiserum absorbed with spores (SAs)

The SAs failed to agglutinate either amoebae or spores (Table III).

Mature spore normal (pre-injection) serum (SN)

The SN used as controls in all agglutination tests along with SA, SAa, and SAs did not effect agglutination of either amoebae or spores (Tables II, III) (Fig. 1b, d).

Mature spore surface antiserum (SSA)

The SSA effected maximal agglutination of both amoebae and spores (Table II).

TABLE III

Degree of agglutination effected by various absorbed antisera and normal (pre-injection) sera on amoebae and spores

+ = maximum agglutination; ± = minimal agglutination; and − = no agglutination. All sera were diluted to one-half of full strength as a result of the addition of amoebae. The numbers opposite the degree of agglutination values refer to the number of times a particular pattern was observed.

	Type of sera	Amoebae Degree of agglutination			Spores Degree of agglutination*		
		+	±	−	+	±	−
Antisera abs. with amoebae	Migrating			26			10
	Spore			24		11	
	Spore surface			3		3	
Antisera abs. with spores	Migrating			9			4
	Spore			14			4
	Spore surface			7			3
Normal sera	Migrating			34			12
	Spore			38			12
	Spore surface			10			4

* See footnote to Table II.

TABLE IV

Comparison of total N in surface antigens of migrating pseudoplasmodia amoebae and mature sorocarp spores

Cell type	No. of exps.	μg. whole cell N	μg. surface antigen N	$\frac{\mu g.\ surface\ antigen\ N}{\mu g.\ whole\ cell\ N}$	Ratio of antigen N $\frac{Spore}{Amoebae}$
Spores	6	133.3	31.0	0.23	1.5
Amoebae	4	53.9	8.0	0.15	

TABLE V

Comparison of total N precipitated from surface antigen preparations of migrating pseudoplasmodia amoebae and mature sorocarp spores by antibodies

	No. of exp.	Surface antigens obtained from		Ratio of precipitate N $\frac{Spore}{Amoebae}$
		Amoebae	Spores	
μg. N precipitated by spore surface antiserum minus controls (normal serum)	3	6.8	30.0	4.4
μg. N precipitated by migrating pseudoplasmodia antiserum minus controls (normal serum)	3	3.0	8.0	2.7

Mature spore surface antiserum absorbed with amoebae (SSAa)

The SSAa failed to agglutinate amoebae but effected maximum agglutination of spores (Table III).

Mature spore surface antiserum absorbed with spores (SSAs)

The SSAs failed to agglutinate either amoebae or spores (Table III).

Mature spore surface normal (pre-injection) serum (SSN)

The SSN did not effect agglutination of either amoebae or spores (Tables II, III).

The amounts of surface material removed from cells composing migrating pseudoplasmodia and mature spores

It was found that in terms of total N of the surface washes, 1.5 times more material was washed from the cells composing the spore masses than from the amoebae composing migrating pseudoplasmodia (Table IV). It was assumed that the N content of the surface washes would serve as an indicator of the amount of antigenic or surface material which had been removed from the cells.

When surface washes of the two types of cells were precipitated with antisera the precipitates obtained from spore surface wash proved to be 2.7–4.4 times greater than those from amoebae surface wash (Table V). This indicates that more antigenic material was removed from the cells composing spore masses than from those composing migrating pseudoplasmodia.

Discussion

Antisera produced in rabbits to antigens composed of cells and washings of cells from various stages of the slime mold, *D. discoideum*, effect agglutination of amoebae and spores. Analysis of the pattern of agglutination effected by the various types of sera on amoebae and spores demonstrated that qualitatively identical surface antigens are present during the transition from the beginning aggregate to the mature spore. However, absorption of various antisera by amoebae and spores has disclosed the presence of a surface antigen(s) in the spores which is (are) not common to early aggregates or migrating pseudoplasmodia. It is possible that the new antigen is composed of the polysaccharide with which spores become coated during the culmination process (Raper and Fennell, 1952).

An examination of Figure 1 reveals that the maximal degree to which amoebae may be agglutinated differs from that degree to which spore cells may be agglutinated. It may be seen that spores agglutinate poorly even with mature spore antiserum as compared with the agglutination of amoebae by the same antiserum.

Shaffer (1958) has demonstrated that *D. discoideum* amoebae become strongly adhesive at the onset of aggregation. Furthermore, it has been observed during the course of the present work that the amoebae composing a migrating pseudoplasmodium do not spread readily upon a glass rod. The failure to spread has been interpreted as being due to the adherence of the cells to each other. However, the cells composing a spore mass tend to spread readily when tested in the same fashion, suggesting that the adhesive properties of the spores are relatively poor.

In seeking an explanation for the particular serological and adhesive properties exhibited by the amoebae and spore cells it was found that surface materials obtained by washing cells in 0.5% NaCl were antigenic (ring and precipitin tests) with MA and SA. Such preparations were subjected to two types of analyses to compare quantitatively the amounts of material which had been extracted from the amoebae and spore cell surfaces. These determinations revealed that greater amounts of antigenic material were found in spore wash as compared to amoebae wash (Tables IV, V). To account for the difference in the amount of antigenic material recovered from surface washings of amoebae and spores it is suggested that an increase occurs in the amount of loosely bound surface antigen on the spores. Another possibility would include an elimination of the antigens from the pre-stalk and pre-spore surfaces as a consequence of their role in morphogenetic movements during the culmination process. Antigens ejected from the cell surfaces would be contained in the spore extracellular spaces. A mechanism by which surface antigens are discharged and renewed at the surfaces of the cells would result in the reversibility of cell adhesiveness. Such reversibility is conceivably necessary if cells in contact with each other are to retain their motility.

Upon injecting spore washings into rabbits, antiserum was produced which was capable of agglutinating both amoebae and spores (Table II). Such antiserum (SSA) when subjected to absorption procedures with amoebae or spores reacted with test cells in the same way as the antiserum (SA) produced by injecting whole spores into rabbits (Tables II, III).

The fact that antiserum obtained from whole spore injection cannot be distinguished from spore surface wash antiserum suggests that the same antigens were involved in the production of each antiserum. The ease with which spore surface wash antigen may be removed implies that it is either loosely bound to the cell surfaces or was discharged from the cells during morphogenesis. Therefore, it seems plausible to attribute the poor agglutination of the spores to the inability of the antigens, with which the antibodies combine, to remain attached to the cell surfaces. Consequently, a reduction in the number of antigenic sites involved in effecting agglutination of cells in the presence of antisera would occur. Few antigenic sites on the spore surfaces would probably suffice for the production of antisera in rabbits to those particular antigens, which accounts for the agglutinating ability of the spore antiserum (SA) on amoebae or spores. Another possibility exists that the poor agglutination of the spores might be attributed to the relative inaccessibility of antigenic sites on spore surfaces. Such an interpretation, for the failure of ox red cells to agglutinate in the presence of certain antisera, was advanced by Coombs *et al.* (1951). The existence of this type of cell surface configuration of the spores could conceivably be tested by Coombs *et al.*'s method.

The poor agglutinating capacity of spores is paralleled by weak adhesiveness between the spores. It is interesting to point out that relatively large amounts of antigen appear in washings of spore cell surfaces. These facts suggest that the particular agglutinating and adhesive properties of the spores are related to the appearance of antigen in spore washings.

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Summary

1. Methods are described whereby antigenic material of the slime mold, *D. discoideum*, effective in antibody production in rabbits, is prepared.

2. Antisera produced in response to migrating pseudoplasmodia, mature spores and mature spore surface antigens effected maximal agglutination of amoebae. Such antisera effected only minimal agglutination of spores.

3. Antisera of the above types absorbed by amoebae and spores in all combinations revealed the presence of a surface antigen(s) on the spores which is (are) not common to amoebae from early aggregates or migrating pseudoplasmodia.

4. It has been suggested that a relationship exists between the adhesive properties of the amoebae and spores and their agglutinating properties in the presence of antisera.

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